

WHAT IS CLAIMED IS:

1. A portable DNA sequence comprising a series of nucleotides capable of directing intracellular production of metalloproteinase inhibitors.

2. The portable DNA sequence of claim 1 wherein said sequence is capable of directing intracellular production of collagenase inhibitors.

3. The portable DNA sequence of claim 1 wherein said nucleotide sequence is:

10	20	30	40	50	60
GTTGTTGCTG TGGCTGATAG CCCCAGCAGG GCCTGCACCT GTGTCCCACC CCACCCACAC					
70	80	90	100	110	120
ACGGCCTTCT GCAATTCCGA CCTCGTCATC AGGGCCAAGT TCGTGGGGAC ACCAGAAAGT					
130	140	150	160	170	180
AACCAGACCA CCTTATACCA GCGTTATGAG ATCAAGATGA CCAAGATGTA TAAAGGGTT					
190	200	210	220	230	240
CAAGCCTTAG GGGATGCCGC TGACATCCGG TTCGTCTACA CCCCCGCCAT GGAGAGTGT					
250	260	270	280	290	300
TGCGGATACT TCCACAGGTC CCACAACCGC AGCGAGGAGT TTCTCATTGC TGGAAAAC					
310	320	330	340	350	36
CAGGATGGAC TCTTGCACAT CACTACCTGC AGTTTCGTGG CTCCCTGGAA CAGCCTGAG					
370	380	390	400	410	42
TTAGCTCAGC GCCGGGGCTT CACCAAGACC TACACTGTTG GCTGTGAGGA ATGCACAGT					
430	440	450	460	470	48
TTTCCCTGTT TATCCATCCC CTGCAAACGT CAGAGTGGCA CTCATTGCTT GTGGACGGA					
490	500	510	520	530	54
CAGCTCCTCC AAGGCTCTGA AAAGGGCTTC CAGTCCCGTC ACCTTGCCTG CCTGCCTCG					
550	560	570	580	590	60
GAGCCAGGGC TGTGCACCTG GCAGTCCCTG CGGTCCCAGA TAGCCTGAAT CCTGCCCGG					

610	620	630	640	650	660
GTGGAAGCTG AAGCCTGCAC AGTGTCCACC CTGTTCCCAC TCCCCATCTTT CTTCCGGACA					
670	680	690	700		
ATGAAATAAA GAGTTACCAAC CCAGAAAAAA AAAAAAGGAA TTC					

4. The portable DNA sequence of claim 2 wherein said sequence is capable of directing intracellular production of a collagenase inhibitor biologically equivalent to that isolable from human skin fibroblasts.

5. A recombinant-DNA cloning vector comprising a nucleotide sequence capable of directing intracellular production of metalloproteinase inhibitors.

6. The vector of claim 5 wherein said vector comprises a nucleotide sequence containing at least the following nucleotides:

10	20	30	40	50	60
GTTGTTGCTG TGGCTGATAG CCCCAGCAGG GCCTGCACCT GTGTCCCACC CCACCCACAG					
70	80	90	100	110	120
ACGGCCTTCT GCAATTCCGA CCTCGTCATC AGGGCCAAGT TCGTGGGGAC ACCAGAACGTC					
130	140	150	160	170	180
AACCAGACCA CCTTATACCA GCGTTATGAG ATCAAGATGA CCAAGATGTA TAAAGGGTTC					
190	200	210	220	230	240
CAAGCCTTAG GGGATGCCGC TGACATCCGG TTCGTCTACA CCCCCGCCAT GGAGAGTGTG					
250	260	270	280	290	300
TGC GGATACT TCCACAGGTC CCACAACCGC AGCGAGGAGT TTCTCATTGC TGGAAAATG					
310	320	330	340	350	360
CAGGATGGAC TCTTGCACAT CACTACCTGC AGTTTGTGG CTCCCTGGAA CAGCCTGAGC					
370	380	390	400	410	420
TTAGCTCAGC GCCGGGGCTT CACCAAGACC TACACTGTG GCTGTGAGGA ATGCACAGTG					
430	440	450	460	470	480
TTTCCCTGTT TATCCATCCC CTGCAAAC TGAGGTGGCA CTCATTGCTT GTGGACGGAC					

490	500	510	520	530	540
CAGCTCCCTCC	AAGGCTCTGA	AAAGGGCTTC	CAGTCCCGTC	ACCTTGCCTG	CCTGCCTCGC
550	560	570	580	590	600
GAGCCAGGGC	TGTGCACCTG	GCAGTCCCTG	CGGTCCCAGA	TAGCCTGAAT	CCTGCCCGGA
610	620	630	640	650	660
GTGGAAGCTG	AAGCCTGCAC	AGTGTCCACC	CTGTTCCCAC	TCCCATCTTT	CTTCCGGACG
670	680	690	700		
ATGAAATAAA	GAGTTACCAAC	CCAGCAAAAA	AAAAAAAGGAA	TTC	

7. The vector pUC9-F5/237P10.

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8. A recombinant-DNA method for microbial production of a metalloproteinase inhibitor comprising:

- (a) preparation of a portable DNA sequence capable of directing a host microorganism to produce a protein having metalloproteinase inhibitor activity;
- (b) cloning the portable DNA sequence into a vector capable of being transferred into and replicating in a host microorganism, such vector containing operational elements for the portable DNA sequence;
- (c) transferring the vector containing the portable DNA sequence and operational elements into a host microorganism capable of expressing the metalloproteinase inhibitor protein;
- (d) culturing the host microorganism under conditions appropriate for amplification of the vector and expression of the inhibitor; and
- (e) in either order:
 - (i) harvesting the inhibitor; and
 - (ii) causing the inhibitor to assume an active, tertiary structure whereby it possesses metalloproteinase inhibitor activity.

9. The method of claim 8 wherein said metalloproteinase inhibitor is collagenase inhibitor.

10. The method of claim 8 wherein said portable DNA sequence is:

10 20 30 40 50 60
GTTGTTGCTG TGGCTGATAG CCCCAGCAGG GCCTGCACCT GTGTCCCACC CCACCCACAG
70 80 90 100 110 120
ACGGCCTTCT GCAATTCCGA CCTCGTCATC AGGGCCAAGT TCGTGGGGAC ACCAGAAGTC
130 140 150 160 170 180
AACCAGACCA CCTTATACCA GCGTTATGAG ATCAAGATGA CCAAGATGTA TAAAGGGTTC
190 200 210 220 230 240
CAAGCCTTAG GGGATGCCGC TGACATCCGG TTCGTCTACA CCCCCGCCAT GGAGAGTGTG
250 260 270 280 290 300
TGC GGATACT TCCACAGGTC CCACAACCGC AGCGAGGAGT TTCTCATTGC TGGAAAATG
310 320 330 340 350 360
CAGGATGGAC TCTTGACAT CACTACCTGC AGTTTCGTGG CTCCCTGGAA CAGCCTGAGC
370 380 390 400 410 420
TTAGCTCAGC GCCGGGGCTT CACCAAGACC TACACTGTTG GCTGTGAGGA ATGCACAGTG
430 440 450 460 470 480
TTTCCCTGTT TATCCATCCC CTGCAAAC TG CAGAGTGGCA CTCATTGCTT GTGGACGGAC
490 500 510 520 530 540
CAGCTCCTCC AAGGCTCTGA AAAGGGCTTC CAGTCCCCTC ACCTTGCCTG CCTGCCTCGG
550 560 570 580 590 600
GAGCCAGGGC TGTGCACCTG GCAGTCCCTG CGGTCCCAGA TAGCCTGAAT CCTGCCCGGA
610 620 630 640 650 660
GTGGAAGCTG AAGCCTGCAC AGTGTCCACC CTGTTCCCAC TCCCATCTT CTTCCGGACA
670 680 690 700
ATGAAATAAA GAGTTACAC CCAGCAAAAA AAAAAAGGAA TTC

11. The method of claim 8 wherein said vector containing said portable DNA sequence is pUC9-F5/237P10.

12. The method of claim 8 wherein said host microorganism is a bacterium.

13. The method of claim 12 wherein said bacterium is a member of the genus Bacillus.

14. The method of claim 13 wherein said bacterium is Bacillus subtilis.

15. The method of claim 12 wherein said bacterium is Escherichia coli.

16. The method of claim 12 wherein said bacterium is a member of the genus Pseudomonas.

17. The method of claim 16 wherein said bacterium is Pseudomonas aeruginosa.

18. The method of claim 8 wherein said host microorganism is a yeast.

19. The method of claim 8 wherein said yeast is Saccharomyces cerevisiae.

20. The method of claim 8 wherein said inhibitor is harvested prior to being caused to assume said active, tertiary structure.

21. The method of claim 8 wherein said inhibitor is caused to assume said active, tertiary structure prior to being harvested.

22. A metalloproteinase inhibitor which is biologically equivalent to the collagenase inhibitor isolable from human skin fibroblasts produced by the method of claim 8.

23. The microorganism C600/pUC9-F5/237P10 having ATCC Accession No. 53003.

24. The portable DNA sequence of claim 1 wherein said nucleotide sequence is:

10 20 30 40 50 6
GGCCATGCC GCAGATCCAG CGCCCAGAGA GACACCAGAG AACCCACCAT GGCCCCCTT

70 80 90 100 110 12
GACCCCTGGC TTCTGCATCC TGTTGTTGCT GTGGCTGATA GCCCCAGCAG GGCCTGCAC

130 140 150 160 170 18
TGTGTCCCCAC CCCACCCACA GACGGCCTTC TGCAATTCCG ACCTCGTCAT CAGGGCCAA

190 200 210 220 230 24
TTCGTGGGA CACCAGAAAGT CAACCAGACC ACCTTATACC AGCGTTATGA GATCAAGATC

250 260 270 280 290 300
ACCAAGATGT ATAAAGGGTT CCAAGCCTTA GGGGATGCCG CTGACATCCG GTTCGTCTAC

310 320 330 340 350 360
ACCCCCGCCA TGGAGAGTGT CTGCGGATAC TTCCACAGGT CCCACAACCG CAGCGAGGAC

370 380 390 400 410 420
TTTCTCATTG CTGGAAAAGT GCAGGATGGA CTCTTGACACA TCACTACCTG CAGTTTCGTC

430 440 450 460 470 480
GCTCCCTGGA ACAGCCTGAG CTTAGCTCAG CGCCGGGGCT TCACCAAGAC CTACACTGTT

490 500 510 520 530 540
GGCTGTGAGG AATGCACAGT GTTCCCTGT TTATCCATCC CCTGCAAACCT GCAGAGTGGC

550 560 570 580 590 600
ACTCATTGCT TGTGGACGGA CCAGCTCCTC CAAGGCTCTG AAAAGGGCTT CCAGTCCCGT

610 620 630 640 650 660
CACCTTGCCT GCCTGCCTCG GGAGCCAGGG CTGTGCACCT GGCAGTCCCT GCGGTCCCAG

670 680 690 700 710 720
ATAGCCTGAA TCCTGCCCGG AGTGGAAAGCT GAAGCCTGCA CAGTGTCCAC CCTGTTCCCA

730 740 750 760 770 780
CTCCCACATT TCTTCCGGAC AATGAAATAA AGAGTTACCA CCCAGCAAAA AAAAAAAGGA